

The inventors have discovered that the present method is particularly good for the differential diagnosis of ulcerative colitis.

The cited reference contains no disclosure or suggestion of such a method. Moreover, this reference contains no teaching which would suggest the effectiveness of the presently claimed method for the differential diagnosis of ulcerative colitis. Accordingly, this reference cannot affect the patentability of the present claims.

The rejection of Claim 14 under 35 U.S.C. § 102(b) in view of (Danielsson et al) is respectfully traversed. Only an abstract of Danielsson et al has been provided, and it discloses: "Two aerobic bacterial species . . . and four strict anaerobic bacterial species including . . . *F. varium* . . . were isolated from fistula secretion of the patient." However, the cited reference is concerned with a patient suffering from Crohn's disease and is completely silent in regard to ulcerative colitis. Accordingly, the presently claimed invention is not anticipated by the cited reference.

Moreover, as explained on page 4, lines 5 to 11, of the present specification:

Fusobacterium varium is a bacterium belonging to the genus *Fusobacterium* of the family *Bacteroidaceae* and was detected from the mucosa of a patient with ulcerative colitis at the active phase by bacterial cell culture and immunostaining. The *rates of these detections were significantly higher than those observed* for patients with ulcerative colitis at the remission stage, *Crohns disease*, ischemic colitis, and colomic adenoma.

The presently claimed invention has been completed on this finding. Thus, by means of the method of Claim 16, the differential diagnosis of ulcerative colitis can be easily made as opposed to cases of inflammatory bowel disease, even though it is usually difficult to distinguish ulcerative colitis from inflammatory bowel disease. There is no teaching in the cited reference which would suggest this result. Accordingly, the presently claimed method is not obvious in view of the cited reference.

For these reasons, the rejection should be withdrawn.

The rejection of Claim 14 under 35 U.S.C. §112, first paragraph is respectfully traversed. In the Official Action, the position is taken that "The specification fails to teach how a sample is obtained? How to determine the amount of antibody significant to make a diagnosis of ulcerative colitis? How to assure that the target antibody (i.e., *Fusobacterium varium*) is obtained and not a mixture of antibodies from other colonic bacteria? Nor does the specification provide a correlation between how to diagnosis of ulcerative colitis and the detection of *Fusobacterium varium* antibodies." (see page 6, line 3 from the bottom to page 7, line 3).

However, Applicants submit that those skilled in the art could easily carry out the above-mentioned points. Thus, the skilled artisan could readily carry out the method of Claim 16 by using either a western blotting method or an enzyme-linked immunosorbent assay (ELISA). Furthermore, as taught in Example 1 of the present specification, *Fusobacterium varium* can be isolated and an antibody specific thereto can be obtained. Accordingly, the present specification and claims meet the requirements prescribed by 35 U.S.C. § 112.

Accordingly, the rejection should be withdrawn.

The rejection of Claim 14 under 35 U.S.C. §112, second paragraph, has been obviated by appropriate amendment. As the Examiner will note, Applicants have rewritten Claim 14 as new Claim 16 to insert steps (a) and (b). Accordingly, the rejection is no longer tenable and should be withdrawn.

The objection to the specification has also been obviated by appropriate amendment. As the Examiner will note, Applicants have amended the specification such that is free of the criticisms outlined on page 3 of the Official Action. Thus, the objection should be withdrawn.

Applicants submit that the application is now in condition for allowance, and early notification of such action is earnestly solicited.

Respectfully submitted,

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A handwritten signature in black ink, appearing to read 'Norman F. Oblon', written in a cursive style.

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IN THE SPECIFICATION

Please amend the specification as follows:

Page 1, line 27, to page 4, line 2, please amend to read as follows:

--Accordingly, it is an object of the present invention to provide a pharmaceutical composition for curing a patient with ulcerative colitis by eradication of [Fusobacterium varium (F. varium)] Fusobacterium varium (F. varium).

It is another object of the present invention to provide a method for screening a drug and treatment having an effect of eradication of [F. varium] F. varium and treating ulcerative colitis, a method for treating a patient with ulcerative colitis, a prophylactic drug for the disease with [F. varium] F. varium infection, a method for preventing any infection with [Fusobacterium varium] Fusobacterium varium, a vaccine for the disease, an agent and a method for diagnosing the infection with the bacterium, and an experimental model and an experimental animal for the disease.

The present inventors have examined patients with ulcerative colitis, and found that [Fusobacterium varium] Fusobacterium varium cells are adhered to mucosa and invade into the mucus and ulcerative mucosa at high rates, the detection rate and value of the serum to the bacterium antibody is high, the amount thereof is large as compared with those observed for normal persons and patients suffering from other intestinal diseases and that the colic ulcer is caused due to butyric acid which is found to be produced by [Fusobacterium varium]

Fusobacterium varium. The [F. varium] F. varium itself has not been recognized to have any pathogenicity and have thus completed the present invention on the basis of the foregoing findings.

Accordingly, the present invention provides a pharmaceutical composition for treating a patient with ulcerative colitis, which comprises a drug capable of selectively killing [Fusobacterium varium] Fusobacterium varium.

The present invention also provides a pharmaceutical composition for treating a patient with ulcerative colitis comprising a drug which can neutralize the toxin produced by [Fusobacterium varium] Fusobacterium varium, a drug which can inhibit the adhesion of [Fusobacterium varium] Fusobacterium varium to the intestinal mucosa or a drug which can inhibit the invasion of [Fusobacterium varium] Fusobacterium varium into the intestinal mucosa as well as a vaccine for preventing ulcerative colitis comprising either of the foregoing drugs in an amount sufficient for preventing [F. varium] F. varium infection.

The present invention likewise provides a method for screening a drug for selectively killing [Fusobacterium varium] Fusobacterium varium, which comprises the step of administering a candidate drug in an experimental model of [Fusobacterium varium-infection] Fusobacterium varium-infection to thus select a drug capable of selectively killing the bacterium.

The present invention also provides a method for screening a therapeutic agent for ulcerative colitis, which comprises the step of administering a candidate drug capable of selectively killing the bacterium to a patient suffering from ulcerative colitis and positive to [Fusobacterium varium] Fusobacterium varium to thus select a drug capable of improving the symptoms of the patients with the disease.

Although [F. varium] F. varium as [commersal] a commercial bacteria has not been recognized to be pathogenic, we found that colonic ulcers indued by enema of butyric acid which was produced by the bacterium.

The present invention provides an animal as a model of ulcerative colitis obtained by administering a toxin produced by [Fusobacterium varium] Fusobacterium varium to an animal.

The present invention also provides an animal as a model for ulcerative colitis comprising [Fusobacterium varium] Fusobacterium varium cells adhered to the intestinal mucosa.

The present invention also provides a method for diagnosing ulcerative colitis comprising the step of detecting an antibody against [Fusobacterium varium] Fusobacterium varium.

The present invention further provides an agent for making a diagnosis of ulcerative colitis comprising an antibody, which has the highest detection sensitivity and the highest specificity, among a plurality of antibodies against [Fusobacterium varium] Fusobacterium varium.--

Page 4, line 5, to page 6, line 28, please amend to read as follows:

--[Fusobacterium varium] Fusobacterium varium is a bacterium belonging to the genus Fusobacterium of the family Bacteroidaceae and was detected from the mucosa of a patient with ulcerative colitis at the active phase by bacterial cell culture and immunostaining. The rates of these detections were significantly higher than those observed for patients with ulcerative colitis at the remission stage, Crohn's disease, ischemic colitis, and [colomic]

colonic adenoma. Therefore, this bacterium is considered to be a causal bacterium or an exacerbation factor. From such information, it would be concluded that the patients with ulcerative colitis could be cured by administering a drug selectively killing this bacterium or an antimicrobial agent to remove the bacterial cells from the mucosa in the lesions. More specifically, there can be used antimicrobial agents having high susceptibility to [Fusobacterium varium] *Fusobacterium varium*, such as tetracycline, penicillin, MNZ, imipenem, amoxicillin, cefmetazole, ampicillin, fosfomycin and chloramphenicol.

It has been recognized that the toxins produced by [Fusobacterium varium] *Fusobacterium varium* cells have toxicity to vero cells. Therefore, the toxins were analyzed and it was found that they were organic acids produced by [Fusobacterium varium] *Fusobacterium varium* and that the principal component thereof was identified to be butyric acid. Thus, when butyric acid was injected into the rectum of a mouse, a lesion similar to ulcerative colitis was induced. Accordingly, if administering a drug, which can neutralize and/or adsorb the butyric acid to a patient, the development of any lesion may be prevented or such conditions may be treated. Specifically, an agent for adsorbing organic acids such as activated carbon can be employed.

Since [Fusobacterium varium] *Fusobacterium varium* cells are adhered to the surface mucosa of active ulcerative colitis, the crisis of ulcerative colitis can be prevented by inhibiting the adhesion of [Fusobacterium varium] *Fusobacterium varium* cells to the affected mucosa even if [Fusobacterium varium] *Fusobacterium varium* cells are present in the intestinal flora. Specifically, a drug for protecting mucosa such as sucralfate and ecabe sodium can be used.

As an evidence that [Fusobacterium varium] Fusobacterium varium cells invade from digestive tracts into the intestinal mucosa, an antibody (serum antibody) against [Fusobacterium varium] Fusobacterium varium is detected in the sera of the patients with ulcerative colitis in a high detection rate and high antibody titers by the ELISA assay. There has recently been elucidated the invasion mechanism of various kinds of bacteria into mucosa. Bacteria act on the mucosal cells by themselves to thus make the cells produce receptors and then they invade into the cells. [Fusobacterium varium] Fusobacterium varium cells may possess the same function. Moreover, it would be considered that if [Fusobacterium varium] Fusobacterium varium cells invade into the mucosa, macrophages can be activated and they may be involved in the crisis and exacerbation of inflammation. Therefore, the inflammation can be controlled by blocking the process of the bacterial invasion into the mucosa.

If a vaccine against [Fusobacterium varium] Fusobacterium varium is developed, it would be possible to immediately eliminate [Fusobacterium varium] Fusobacterium varium cells even if infected therewith and to prevent any crisis of ulcerative colitis or to inhibit the exacerbation of the symptoms, as in the case where the infection with pneumococcus can be inhibited by the vaccination with a pneumococcal vaccine.

A target drug can be screened by establishing an experimental infection model using a sterilized animal or a small animal in which [Fusobacterium varium] Fusobacterium varium cells are adhered to the colonic mucosa. In these animals, a candidate drug administered to the experimental animal, can be evaluated whether the candidate drug can selectively kill [Fusobacterium varium] Fusobacterium varium cells or not, by the culture, immunostaining and the serum antibody titer.

Patients with ulcerative colitis infected with [Fusobacterium varium] Fusobacterium varium [with] which are identified by diagnosing patients suffering from ulcerative colitis through the determination of the serum antibody titers, the cultivation of the mucosa or the immunostaining of affected mucosa, and then a candidate drug capable of selectively killing [Fusobacterium varium] Fusobacterium varium is administered to the patients after obtaining the informed consent of patients. Thus, the effectiveness of the drug as a therapeutic agent for ulcerative colitis can be evaluated by the symptoms of the ulcerative colitis are, in fact, improved or not and by investigating the presence of side effects and safety of the drug.

Moreover, the principal component of the toxin against the vero cells produced by [Fusobacterium varium] Fusobacterium varium is found to be butyric acid, and butyric acid is injected into the colon of a small animal such as a mouse or a rat to thus establish a lesion similar to ulcerative colitis.

[Fusobacterium varium] Fusobacterium varium cells are inoculated to germ-free animal or [an] a compromised small animal and [adhere] the bacterial cells adhere to the colonic mucosa. Moreover, it is also possible to find out animals highly sensitive to the adhesion of living bacterial cells to the intestinal mucosa by the inoculation of [Fusobacterium varium] Fusobacterium varium cells into a variety of animals. Thus, an ulcerative colitis-like lesion can be developed or established in these animals and the establishment of such a lesion in the animals or the infection thereof with [Fusobacterium varium] Fusobacterium varium can be judged on the basis of the results of the detection of the serum antibody, the immunostaining of mucosa and culture of the mucosa.--

Page 7, lines 10-25, please amend to read as follows:

--The pharmaceutical composition of treatment for ulcerative colitis according to the present invention comprises a drug capable of selectively killing [*Fusobacterium varium*] *Fusobacterium varium*, a drug, which can neutralize the toxin produced by [*Fusobacterium varium*] *Fusobacterium varium*, a drug, which can inhibit the adhesion of [*Fusobacterium varium*] *Fusobacterium varium* to the mucosa or a drug, which can inhibit the invasion of [*Fusobacterium varium*] *Fusobacterium varium* present into the mucosa, alone or in any combination with a variety of other additives. The resulting composition is thus ready for administering to a patient with ulcerative colitis and positive to infection with [*Fusobacterium varium*] *Fusobacterium varium*. Examples of the dosage forms of such a pharmaceutical composition include tablets, powders, pills, granules, capsules, suppositories, solutions, sugar-coated tablets, depots, or syrups. These dosage forms may be prepared by the use of currently used pharmaceutical auxiliary agents according to the usual methods. In this case, the pharmaceutical compositions preferably comprise pharmaceutically acceptable carriers and/or diluents in addition to the foregoing drugs.--

Page 8, lines 23, to page 9, line 23, please amend to read as follows:

--Example 1

Biopsy specimens from patients with active UC were cultured aerobically and anaerobically. Bacteria with filtered culture supernatants cytotoxic to Vero cells were examined by PCR for verotoxin genes. HPLC was performed with culture supernatants of cytotoxic strains for organic acid concentrations. Mice were given enemas containing organic acid at mean concentration in the supernatants of cytotoxic strains to ascertain whether colonic lesions resembling those of UC appeared. We further investigated sera of patients

with UC for antibodies against cytotoxic bacteria by western blotting and enzyme-linked immunosorbent [assays(ELISA)] assays (ELISA), and examined biopsied mucosa for these bacteria by immunohistochemistry. Only strains of [Fusobacterium varium(F. varium)] Fusobacterium varium (F. varium) isolated killed Vero cells. The bacterium lacks verotoxin genes but culture supernatant was found to contain much butyric acid. Twenty-four hours after enemas containing butyric acid or [F. varium] F. varium culture supernatants, mice demonstrated colonic ulcers with crypt abscesses, inflammatory cell infiltration and apoptotic changes. Western blotting with [F varium] F. varium showed 19 of 31 (61%) patients to have serum antibodies to the bacterium. In [ELIZA] ELISA and immunohistochemistry with [F varium] F. varium proteins an antigen, the mean optical density and the detection rate were higher for our patients than for subjects with Crohn's disease or other controls.

Example 2

The susceptibility to [Fusobacterium varium] Fusobacterium varium in antimicrobial agents was examined. As a result, [Fusobacterium varium] Fusobacterium varium was found to be [susceptibility] susceptible to the following antimicrobial agents in the order indicated: tetracycline > penicillin > MNZ > imipenem > amoxicillin, cefmetazole, ampicillin, fosfomycin, chloramphenicol. On the other hand, the bacterium was found to be resistant to clarithromycin, erythromycin, streptomycin, kanamycin and gentamycin.--

IN THE CLAIMS

Please cancel Claim 14, without prejudice toward the further prosecution of this claim in a Continuation and/or Divisional Application.

Please add the following new claims:

--16. (New) to 18. (New)--